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(54) STABILIZATION OF COLLOIDAL SYSTEMS BY THE FORMATION OF IONIC LIPID-POLYSACCHARIDE COMPLEXES

(57) Stabilization of colloidals systems through the formation of ionic lipid-polisaccharide complexes. There is disclosed a process for the preparation of colloidal systems which includes the incorporation of a water soluble and positively charged amino polysaccharide and a negatively charged phospholipid. The colloidal systems (which comprise polymer nanoparticles, nanocapsules and nano-emulsions) are stabilized through the formation of a ionic complex, at the interface, comprised of the aminopolysaccharide and the phospholipid. The colloidal systems are characterized in that they have a particle size lower than 1 μm, a electric positive charge and an exeptional stability during storage. They are lyophilizable so that they can be dry stored and redispersed subsequently while maintaining the original characteristics of the system. They are useful as pharmaceutical forms for the oral, transdermic, topical, ocular, nasal and vaginal administration of medicaments. They are also useful as forms for cosmetic use.

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Description

Stabilization of colloidal systems through the formation of lipid-polyssacharide complexes. Development of a procedure for the preparation of colloidal systems involving a combination of two ingredients: a water soluble and positively charged polyssacharide and a negatively charged phospholipid. The procedure can be applied to the stabilization of colloidal systems of pharmaceutical and cosmetic use. These systems include oil-in-water submicron emulsions, nanocapsules consisting of an oily core surrounded by a polymer coating and polymeric solid nanoparticles. The common feature to all these colloidal systems is that they consist of a dispersed phase -oily nanodroplets, nanocapsules or nanoparticles-and a continuous aqueous phase. The originality of the procedure relies on the incorporation of lecithin (anionic ingredient), as a lipophilic surfactant, in the dispersed phase and of the chitosan, as a hydrophilic suspending agent, in the continuous aqueous phase.

Lecithin is a natural surfactant composed of a mixture of various phospholipids. The major compound is phosphatidylcholine (phospholipid of a neutral character) and the secondary compounds are phosphatidylethanolamine, phosphatidylserine and phosphatidic acid (phospholipids with a negative charge). Presently, there are several types of lecithin available in the market. They differ in their origin and in their phosphatidylcholine content.

Chitosan is a natural polymer obtained by a deacetatilation process of the chitin (compound extracted from the crustacean shells). Chitosan is a aminopolysaccharide and has a positive charge. Presently, there are several types of chitosan available in the market. They differ in their molecular weight, deacetilation degree and the type of salt or acid form.

The colloidal systems covered in this patent are characterized because they contain lecithin ad chitosan in their composition and they have a positive charge and an improved stability. Other ingredients will be specific of the type of system i.e. an oil, in the case of the submicron emulsions; an oil and a hydrophobic polymer, in the case of the nanocapsules, and a hydrophobic polymer in the case of the nanoparticles. Drugs, proteins and other bioactive compounds of interest in medicine and cosmetics can be incorporated in these systems. Consequently, the application of these systems could be extended to the fields of medicine and cosmetics.

A important draw back of the colloidal carriers is their unstability following in vivo administration and also during storage. It is well known that the majority of the colloidal carriers have a negative surface charge and, because of this fact, they interact with the cationic biologic compounds upon in vivo administration, thus leading to the coalesce and destruction of the system. Difficulties in the freed-drying process, more specifically problems in the reconstitution of the freed-dried systems, represent another important limitation for the correct exploitation of the colloidal systems specially the nanocapsules and submicron emulsions. As a consequence, these systems have to be stored as a suspension liquid form, a situation that normally leads to the destruction of the systems in a few months. The novel systems presented in this patent have a positive charge and an improved stability upon contact with biological cations and during storage. Consequently, these systems overcome the limitations mentioned above.

There are in the literature an important number of publications and patents describing procedures to produce colloidal systems such as nanoparticles, nanocapsules and submicron emulsions. Therefore, the production of these systems is not the object of the present invention. The object is, however, the incorporation in such colloidal systems of two specific ingredients: lecithin ad chitosan. The preparation of these systems involves the use of two phases: a oily phase that is dispersed in an aqueous phase. Both phases normally contain surfactants. The most common surfactant introduced in the oily phase is lecithin. Lecithins are natural compounds that contain phosphatidylcholine and other phospholipids of negative charge. Consequently, colloidal systems containing lecithin have a more or less important negative surface charge. This negative charge normally leads to the destruction of the system, mainly upon contact with biological cations. This limitation inherent to most of the colloidal systems has been recently overcome by using lipophilic surfactants with a positive charge. These positive surfactants are introduced in the dispersed oily phase (S. Benita, oil-in water emulsion of positively charged particles WO 93/18852).

The present invention describes a new approach to provide the colloidal particles of a positive charge. This approach is based on the use of the cationic polyssacharide, chitosan, that is dissolved in the continuous aqueous phase and a lipid anionic surfactant, such as lecithin, that is introduced in the oily dispersed phase. The positively charged chitosan molecules interact with the negatively charged phospholipids, thus forming a film at the interface of the colloidal system. The interaction process of chitosan with phospholipids was previously described as a way to stabilize emulsions (no submicron emulsions) *(P. Faldt, B. Bergenstahl, P.M. Claesson, Stabilization by chitosan of soybean oil emulsions coated with phospholipid and glycocholic acid, Colloids Surfaces A: Physicochem. Eng. Aspects 71,187-195, 1993)* and liposomes *(I. Henriksen, G. Smistad and J. Karlsen, Interactions between liposomes and chitosan, Int. J. Pharm., 101, 227-236, 1994)*. Nevertheless, no reference concerning the application of such interaction (chitosan-phospholipid) to the stabilization of submicron emulsions, nanocapsules and nanoparticles has been found. On the other had, it is important to mention that the approaches described until now for the freeze-drying of colloidal systems, such as nanocapsules and submicron emulsions, are based on the use of enormous amounts of sugars (R.J. Gautier and R.S. Levinson, Lyophilized emulsion compositions and method, South Africa patent No. 864032) whereas the freeze drying of the nanocapsules covered in this invention require the use of relatively low amounts of

sugars (less than 10%).

The systems covered in this patent, characterized by the formation of a polysaccharide-lipid complex at the interface, have some relevant advantages: (1) The systems can be stored in a suspension liquid form for extended periods of time, (2) the nanocapsules based on this approach can be freeze dried and the resultant dry product reconstituted upon addition of water (3) the chitosan-coated nanocapsules herewith described are more stable in the presence of biological cations than conventional uncoated nanocapsules, (4) the systems have a positive electrical surface charge that enables their interaction with negatively charged biological surfaces.

The present invention describes novel systems of interest in therapeutics and cosmetics. These systems can be presented in a liquid form of variable viscosity or in a semi-solid (cream) or solid form (freeze-dried powder).

The dispersed phase of the system consists either of a polymer or an oil or both substances simultaneously. The specific ingredient of this dispersed phase is a negatively charged phospholipid. This phase can contain as well a variable amount of an active ingredient. The oils can be chosen among vegetable oils or semisynthetic polyoxyethylenated oils (Migliol®, Labrafil®, Labrafac®...) of various H.L.B. (hydrophilia lipophilia balance) values. The polymer can be any hydrophobic polymer which is adequate for pharmaceutical or cosmetic use. The proportion of the hydrophobic polymer with respect to the oily phase can vary from 0% (submicron emulsions) up to 100% (nanoparticles). Intermediate proportions lead to the formation of nanocapsules in which the oil is in the polymer forming a reservoir system.

The specific ingredient of the external aqueous phase is chitosan. For freeze drying purposes some cryoprotective agents such as dextran and glucose need to be added to this external phase. This phase can incorporate as well ingredients to provide a certain density or viscosity to the preparation, bacteriostatic agents for the prevention of contamination and other hydrophilic agents.

These systems can be formulated in different ways in order to incorporate in their structure one or more active ingredients of a hydrophilic or lipophilic character. Active ingredient is the ingredient for which the formulation is destined; in other words, the ingredient which will have an effect following its administration to an organism (humans or animals). The corresponding effect can be curing, minimizing or preventing a disease (drugs, vitamins, vaccines...) or improving the physical appearance and aesthetics (e.g... skin hydration...) and other.

Cyclosporin A, an immunossupressive peptide, indomethacin (anti-inflammatory drug) metipranolol (beta-blocker) and tiopental (hypnotic agent) are examples of drugs which have been successfully associated to the colloidal systems described in this patent.

A common feature to the systems described in this patent is the colloidal nature, which means that their size is lower that 1μm. Tables 1 and 2 show the mean particle size of the nanocapsules, submicron emulsions ad nanoparticles containing the oil Migliol® 840 and various amounts of polyepsiloncaprolactone, soybean lecithin and dextran.

As mentioned above, a relevant property of the systems described here is their positive electrical charge. This positive charge promotes the interaction of the systems with the negatively charged mucosa and epithelia and also improves their stability in the presence of biological cations. As shown in table 3 the zeta potential of the systems varies between +30 and +60 mV, being these values dependent on the molecular weight of chitosan.

The inner structure of the systems described here is variable ad dependent upon the composition of the system. As indicated before, the composition of the systems can vary substantially being the common ingredients lecithin and chitosan or their derivatives. Two main inner structures can be described: a reservoir system consisting of a oily core surrounded or not by a polymer wall, and a matrice system consisting of solid particles containing none or little amounts of oil entrapped.

The redispersability of the colloidal systems upon freeze-drying is a major advantage of the systems covered in this patent. Tables 3 and 4 show the particle size of the nanocapsules before and after freeze-drying.

The procedure described in this invention leads to the formation of novel systems for pharmaceutical or cosmetic applications. In addition, these systems could be administered by various routes: topical, oral, nasal, pulmonary, vaginal and subcutaneous. The specific ingredients, chitosan and lecithin, provide to these systems a positive electrical charge and an improved stability not only during storage but also upon freeze-drying and further rehydration.

Table 1

Particle size of the poly(ε-caprolactone) (PECL) nanocapsules containing Migliol ® 840 and a fixed concentration of chitosan (Seacure 123, 0.2%)				
% Lecithin (w/v)	**% Dextran (w/v)**	**% PECL (w/v)**		
		0	**1**	**2**
0,5	1	340 ± 23	361 ± 22	353 ± 21
0,5	2	278 ± 43	324 ± 28	292 ± 38
1	1	324 ± 23	384 ± 5	313 ± 19
1	2	313 ± 11	303 ± 28	318 ± 24
1,5	1	314 ± 19	341 ± 18	346 ± 20
1,5	2	284 ± 12	321 ± 10	339 ± 13

Table 2

Particle size of the poly(ε-caprolactone) (PECL) nanoparticles prepared with a fixed concentration of chitosan (Seacure 223, 0.2%)			
% Lecithin (w/v)	**% Dextran (w/v)**	**% PCL (w/v)**	
		1	**2**
0,5	**1**	290 ± 16	308 ± 15
0,5	**2**	286 ± 12	296 ± 20
1	**1**	330 ± 15	330 ± 2
1	**2**	299 ± 16	317 ± 10
1,5	**1**	337 ± 10	355 ± 19
1,5	**2**	326 ± 18	332 ± 12

Table 3

Zeta potential of the poly(ε-caprolactone) (PECL) nanocapsules and submicron emulsions containing Migliol ® 840 and a fixed concentration of chitosan (Seacure 320, 0.2%).			
% Lecithin (w/v)	**Zeta Potential (mV)**		
	Submicron emulsions	**Nanocapsules**	
		PECL 1%	**PECL 2 %**
0,5	+ 52 ± 2	+ 60 ± 1	+ 60 ± 1
1	+ 60 ± 1	+ 61 ± 1	+ 60 ± 0.07
1,5	+ 59 ± 0.3	+ 59 ± 2	+ 61 ± 0.4

Table 4

Particle size of the poly(ε-caprolactone) (PECL) nanocapsules containing Migliol ® 840 and a fixed concentration of chitosan (Seacure 223 viscosity 100 cps and Seacure 320 viscosity 680 cps, 0.2%). Final concentration of PECL and lecithin in the suspension: 1% and 0.5% respectively.			
Chitosan viscosity	**% Dextran (p/v) (cps)**	**Particle size (nm)**	
		Before freeze- drying	**After freeze- drying**
100	**1**	459 ± 23	487 ± 19
100	**2**	472 ± 8	462 ± 19
680	**1**	443 ± 30	475 ± 30
680	**2**	461 ± 13	505 ± 16

Example 1:

-Preparation of a formulation of nanocapsules containing PECL and Migliol ® 840.
The nanocapsules were prepared using the following ingredients (%, w/w):

Migliol® 840 oil	0.5
Soybean lecithin	1.0
polyepsiloncaprolactone	1.0
Dextran	1.0
Chitosan	0.2
Water	up to 100%

Chitosan and dextran were dissolved in an acidic aqueous solution (acetic acid 0.05M, pH 5.0). The oil Migliol® 840, the surfactant soybean lecithin and the polymer poly(ε-caprolactone) were dissolved in 25 ml of acetone. The acetonic solution was then added, upon magnetic agitation, to an aqueous solution. Three min later the system was transferred to a rotavapor for the elimination of the acetone. The size and zeta potential of the nanocapsules were: 385 nm and +45mV respectively.

Finally, glucose was dissolved in the aqueous suspending medium and the nanocapsules freeze-dried. The particle size and zeta potential of the nanocapsules was determined again upon freeze-drying and resuspension. Results were: 359 nm and +42mV.

Example 2:

--Preparation of a formulation of nanocapsules containing PECL and Migliol ® 840.

The nanocapsules were prepared as described in example 1 but containing different amounts of lecithin and oil::

Migliol® 840 oil	1.5
Soybean lecithin	0.5
polyepsioloncaprolactone	1.0
Dextran	1.0
Chitosan	0.2
Water	up to 100%

The particle size and zeta potential of these nanocapsules were: 433 nm and +32 mV, respectively, before freeze-drying and 582 and +43 mV after freeze-drying.

Example 3:

--Preparation of a formulation of a submicron emulsion containing Migliol ® 840.
The emulsion was prepared as described in example 1 but without the polymer PECL:

Migliol® 840 oil	1.5
Soybean lecithin	0.5
Dextran	1.0
Chitosan	0.2
Water	up to 100%

The results of particle size and zeta potential were: 463 nm and +42 mV, respectively.

Claims

1. Process for the preparation of pharmaceutical compositions in the form of colloidal particles of a size less than 1μm suitable for the delivery of active compounds characterized in that they are coated and stabilized by film made of a combination of a cationic aminopolyssaccharide, soluble in water, and a phospholipid negatively charged.

2. Process according to claim 1, characterized in that the formation and coating of the colloidal particles occurs simultaneously and spontaneously upon mixing two miscible phases, one of them being an organic solution of a phospholipid and other ingredients and the other one being an aqueous solution of an aminopolyssaccaride and other ingredients.

3. Process according to claim 2, characterized in that said aminopolysaccharide is selected from the group consisting of chitosans and derivatives thereof.

4. Process according to claim 2, characterized in that said phospholipid is selected from the group of lecithins and derivatives thereof.

5. Process according to claim 1 to 4, characterized in that the percentage of the aminopolyssacharide in the final aqueous medium can be up to 2% preferably between 0.05 ad 0.5% by weight.

6. Process according to claim 1 to 5, characterized in that the percentage of the phospholipid in the final aqueous medium can be up to 5% preferably between 0.2 and 1% by weight.

7. Process according to claim 1 to 6, characterized in that the colloidal system is a submicron emulsion which is formed upon the incorporation of a vegetable or semisynthetic oil, dissolved in an organic phase, into an aqueous phase, the oil being in an amount up to 1% with respect to the external aqueous medium.

8. Process according to claim 1 to 6, characterized in that the colloidal system is a suspension of nanocapsules which is formed upon the incorporation of a vegetable or semisynthetic oil and a polyester, dissolved in an organic phase, into an aqueous phase, the oil and the polyester being in variable proportions up to 1% and 4% respectively.

9. Process according to claim 1 to 6, characterized in that the colloidal system is a suspension of nanoparticles which is formed upon the incorporation of a polyester, dissolved in an organic phase, into an aqueous phase, the polyester being in variable proportions up to 4%.

10. Process according to claim 1 to 9, characterized in that the composition includes an active ingredient that can be incorporated either into the aqueous or into the organic phase, this active ingredient being in variable proportions with respect to the aqueous medium.

11. Process according to claim 8, characterized in that the composition includes complementary ingredients, in particular dextran (1-2%) and glucose (5%), to allow the freeze-drying of the nanocapsules and further resuspension in water.

12. Process according to claims 1 to 11, characterized in that said active ingredient is selected from the group: indomethacin, metipranolol, diazepam and cyclosporin A.

13. Process according to claims 1 and 2, characterized in that the composition includes ingredients which are non-toxic and compatible with their application by the topical, oral, nasal, vaginal and pulmonary routes of administration. These particles have a positive charge that facilitates their interaction with mucosas and epithelia.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/ES96/00116

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl.6 A61K 47/24, 47/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl.6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CIBEPAT, EPODOC, WPIL, PAJ, CA, MEDLINE, PHAR, BIOSIS, TXTEP1.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP-0486959-A (VECTORPHARMA INTERNATIONAL, S.P.A.) 27.05.92, example 10.	1-6,9,12,13
A	Fäldt, P. et al. "Stabilization by chitosan of soybean oil emulsions coated with phospholipid and glycocholic acid". (1993) COLLOIDS AND SURFACES A: PHYSICOCHEMICAL AND ENGINEERING ASPECTS", vol 71,pp.:187-195, the whole document.	1-13

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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